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Multicolored silica coated CdSe core/shell quantum dots

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ABSTRACT

Silanization is a convenient route to obtain water-soluble quantum dots (QDs) with different structures. Green, orange and red emitting CdSe-based QDs were synthesized by varying the number and the material of the wider-band gap shells and the fluorescent properties of the QDs were characterized before and after silanization. It was shown that the structure of the QD influences the quantum yield of the silanized QDs: the better the CdSe core is protected with wider-band gap semiconductor shells, the more the fluorescence properties remain present after water solubilization. The proposed silanization method allows imparting highly hydrophilic properties to QDs with different emission colors. Hence, silica coated QDs have a great perspective for multiplex analysis.

Key words: quantum dots, silica, quantum yield, structure, multiplex assay

1. INTRODUCTION

Nowadays, the quality of human life is determined by the accuracy of diagnostics, treatment, food control and environmental monitoring. This requires availability of sensitive multiplex assays, which simultaneously measure multiple analytes in a single cycle of the analysis. Fluorescence-based assays, especially combined with immunological techniques, can meet these requirements. A fluorescence analytical method based on quantum dots (QDs) can provide advantages over the use of traditional organic dye molecules in term of sensitivity, stability, and capability for multiplexing detection. Compared to organic dye molecules, QDs have high fluorescence quantum yields (QY), stability against photobleaching, narrow emission bands, high Stokes shifts, broad absorption spectra, allowing usage of one excitation source for QDs with different emission wavelength[1]. These advantages have been widely applied for the multiplexed simultaneous detection of different targets[2-7].

One of the main challenges, encountered in the development of QD-based systems, is the selection of a suitable capping ligand in order to achieve adequate solubility of QDs in the dispersion media. Generally, during their high-temperature synthesis, the QDs acquire a hydrophobic surface layer. As a result, QDs are intrinsically soluble in non-polar media and suitable surface modification must be performed if the dispersant solvent needs to be changed. The transfer of QDs to a polar medium, without changing their optical properties and their ability to interact with target analytes, is a critical step[5].

Encapsulation of hydrophobic QDs into a SiO_2 shell is a well-known approach to make QDs soluble in water[8]. Silica coated nanoparticles (NPs) have several advantages. In the first place, silica may provide both chemical and physical shielding from the direct environment, thereby improving the stability. For example, it can reduce the release of (cytotoxic) ions, prevent photooxidation processes and aggregation of the nanocrystals. Furthermore, the silica shell is highly hydrophilic and the surface chemistry of colloidal silica is well investigated. Therefore, this approach facilitates not only solubilization in different solvents, but it also enhances the bioapplicability and it allows modifications of the composite particles for further use. In addition, silica spheres can contain multiple nanoparticles per particle (e.g., gold, silver, magnetite), and can as such serve as the starting point for the development of a multimodality contrast agent[9].

The aim of the current work was to investigate how the structure (and consequently, the emission color) of the QDs impacts the fluorescent properties of the silica coated QDs (QD@SiO_2), obtained by means of the reverse-microemulsion method, in order to obtain stable and bright multicolor labels.

2. EXPERIMENTAL SECTION

2.1. Chemicals

Cadmium oxide (CdO, 99,99%), selenium powder (Se, 99,99%), sulfur powder (S, 99%), zinc acetate ($Zn(OAc)_2$, 99,99%), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), oleylamine (OLA, 70%), octadecylamine (ODA, 90%), trioctylphosphine (TOP), rhodamine 6G, TEOS, surfactant Brij L4 (M_n~362) were purchased from Sigma-Aldrich (Bornem, Belgium). All organic solvents (ethanol, butanol, toluene, chloroform) and ammonium solution (25%) were purchased from Sigma-Aldrich (Bornem, Belgium) and were used without further purification. All other chemicals and solvents were of analytical grade. Ultrapure Mili Q water was used throughout.

2.2. Synthesis of CdSe core nanocrystals

CdSe QDs were prepared via the well-known the rapid hot-injection method[10,11]. CdSe cores were synthesized as follows: 1 mmol of a Se-precursor solution (0.1 M Se in TOP and ODE) was injected into a three-neck flask containing 1 mmol of Cd-precursor solution (0.1 M CdO in OA and ODE) at 260 °C. The reaction was stopped after 20 s. Next, to isolate the CdSe QDs, a buthanol-ethanol mixture was added and a centrifugation was performed. Finally, the precipitate was dissolved in toluene and stored at 4 °C. The size (2.5 nm) and concentration (8 µM) of the CdSe nanocrystals so obtained were estimated according to the formula presented in [12].

2.3. Synthesis of core/multishell QDs

Green-emitting QDs (λ^{em}=550 nm, QD^{550}) were produced by means of the low-temperature shell growing process as described in [13]. A mixture of Zn-precursor (0.1M $Zn(Ac)_2$ in OLA and ODE), S-precursor (0.1M S in ODE) and ODE was flashed with Ar. Next, a 0.3 µmol toluene solution of CdSe was added to the mixture and the temperature was increased up to 140^0C for 2 h. To purify the CdSe/ZnS QDs from unreacted precursors, acetone was added to the CdSe/ZnS solution (V(QD solution):V(acetone)=1:2). The samples were left for 10 min and then centrifuged at 4000 rpm during 5 min. The precipitate was dissolved in a sufficient amount of toluene.

Core/shell red- and orange-emitting QDs were produced by means of the Successive Ion Layer Adsorption and Reaction (SILAR) technique described in [11] with slight modifications. First, ODE, ODA and 0.3 µmol CdSe cores were loaded into a 100 mL three-neck flask and heated at 100 °C under inert atmosphere for 1 hour. Next, S-precursor (0.1 M S in ODE), Cd-precursor (0.1 M CdO in OA and ODE) and Zn-precursor (0.1M $Zn(Ac)_2$ in OLA and ODE) were successively added, waiting 10 min between each injection at 220°C. Upon completion of the synthesis, the reaction mixture was purified with acetone addition and centrifugation. Finally, the precipitate was dissolved in toluene and stored at 4 °C. By means of this method, orange-emitting QDs with structure CdSe/2CdS/2CdZnS/2ZnS (λ^{em}=600 nm, QD^{600}) and red-emitting QDs with structure CdSe/3CdS/2ZnS (λ^{em}= 630 nm, QD^{630}) were synthesized.

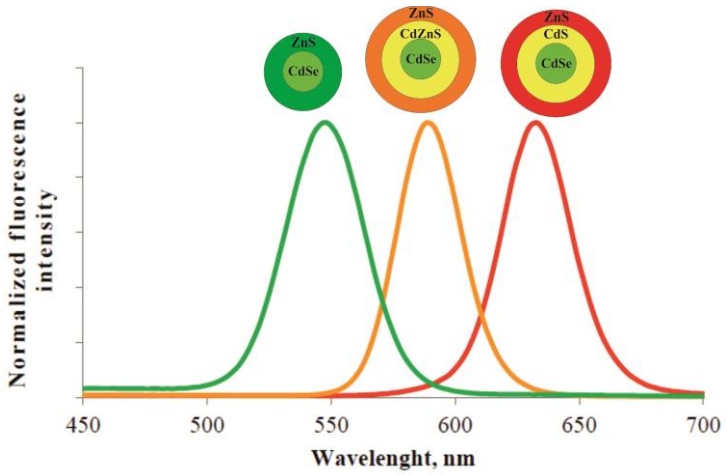

Fig. 1. Emission spectra of the synthesized CdSe-based QDs with: ZnS shells (green line), CdS and ZnS shells (red line), and CdS and ZnS with an alloy CdZnS layer (orange line)

2.4. Synthesis of QD@SiO_2 NPs

The above mentioned QDs (QD^{550}, QD^{600} and QD^{630}) were encapsulated in silica NPs through the microemulsion process[14]. First, 1 ml of hexane, 0.32 ml Brij L4 as surfactant and 50 µl H_2O were mixed and let to stir for 30 min to form the microemulsion. Then, 1 nmol of QDs in chloroform was slowly added. 30 min later, 30 µl of TEOS was added, following by the addition of 10µl NH_3 solution, which acts as the catalyst of the hydrolysis process. Subsequently, the reaction mixture was aged for 24 h at room temperature and the NPs were precipitated from the microemulsion using ethanol, centrifuged and the resultant precipitate of QD@SiO_2 particles were washed 3 times with hexane and at least 5 times with deionized water. Finally, aqueous dispersions of the composite particles were obtained via sonication.

2.5. Characterization techniques

For the relative QY estimation, the spectrally integrated emission of the QDs solutions was compared to the emission of a rhodamine 6G ethanol solution (QY=95% at excitation wavelength 490 nm) of identical optical density (~0.05) at the excitation wavelength[15]. UV-vis absorption spectra of the QDs were measured by a Shimadzu 1800 spectrophotometer. Photoluminescence (PL) spectra were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies).

3. RESULTS AND DISCUSSION

CdSe cores were prepared via a rapid hot-injection method in ODE using TOP as a stabilizer. To improve their fluorescence intensity and stability, CdSe nanocrystals were covered with several shells of the wider band gap semiconductors, such as CdS and ZnS (Fig. 1).

It is well known that ZnS is the best protecting shell for CdSe QDs because of the considerable differences in the values of the band gap energies[16]. Low-temperature (120^{o}C) synthesis was used to cover the CdSe cores directly with ZnS shells, using OA and OLA as stabilizers. This method allows increasing the QY of the QDs (by a factor of 10 on average), while the emission maximum does not significantly shift: 550 nm for the CdSe/ZnS QDs compared to 535 nm for the CdSe cores.

For efficient shell growing it is important to use semiconductors with similar parameters concerning their crystalline lattice. In the case of CdSe and ZnS semiconductors, these parameters are quite different. Thus it is preferably to introduce the additional middle shell (CdS or ZnSe) between the CdSe core and the ZnS outer shell to reduce strain inside the nanocrystal[17]. A high-temperature (230^{o}C) SILAR technique was used to cover the CdSe core with CdS and ZnS shells, using ODA as stabilizer. The difference between the band gaps of CdSe and CdS is not sufficient for a complete confinement of the electrons and holes inside the CdSe core. Therefore, formation of an intermediate CdS shell around the CdSe cores resulting in a red shift of both the absorption and fluorescence maxima. Due to blocking of electrons and holes inside the core, the fluorescent properties improved with each shell. Finally, the fluorescence QY of core-shell particles reached 50%, which is 10 times more than the QY of uncovered CdSe cores (Fig. 2).

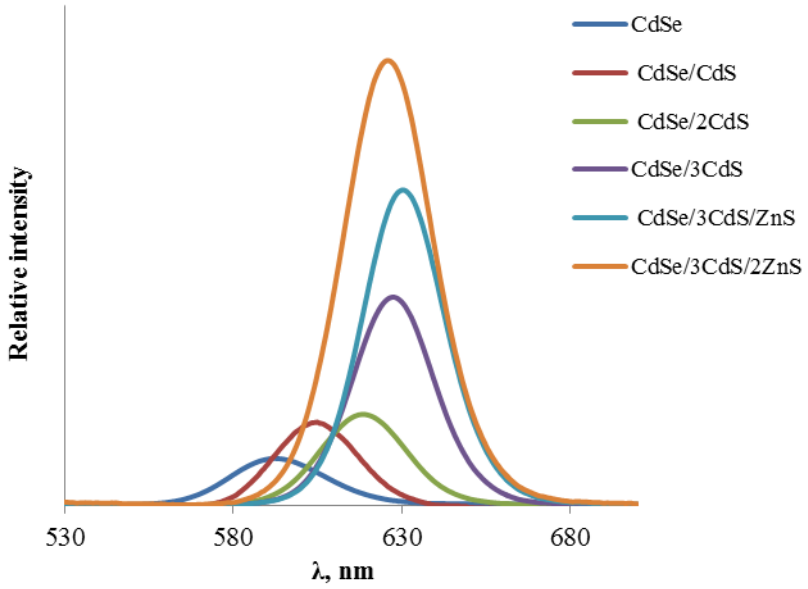

Fig. 2. Emission spectra of CdSe cores with a varying number of CdS and ZnS shells (intensity is based on relative QY values)

The encapsulation of QDs in silica spheres can be achieved through two different methods. The first method is via Stöber synthesis, where the QDs act as seeds for silica growth in an ethanol/water mixture. Typically, this approach is applicable to initially hydrophilic QDs. This method yields single or multiple QDs per silica sphere, but the size and size dispersion of the QD/silica particles are not well-controlled.

The second method implies using a water-in-oil reverse microemulsion system, where small water droplets are stabilized by a nonionic surfactant (e.g., NP-5, Igepal or Brij) in a continuous hydrophobic phase (e.g., cyclohexane, hexane, heptane). Hydrolysis and condensation of the silica precursor (e.g., TEOS) take place in the water phase, resulting in highly monodisperse silica particles, even at small sizes (>25 nm).

The best possible mechanism of silanization in microemulsion assumes a ligand exchange: the initial hydrophobic ligands (TOP or OA) on the QD's surface are exchanged by partly hydrolyzed TEOS molecules. It results in quenching of the fluorescent properties of the QDs, because the hydrolyzed molecule of TEOS can act as an efficient hole or electron acceptor, which introduces a new non-radiative decay pathway for the exciton[9]. Also, the rate of the ligand exchange reaction dramatically affects the fluorescence properties of the final silanized QDs. Thus, purity of TEOS affects the ligand exchange reaction rate, and, consequently, the QY of the silica coated QDs.

Long chain amines as OLA and ODA were used as a stabilizer, because they are found to be more suitable surfactants for the semiconductor nanocrystals[17]. This has been attributed to the close packing (theoretically, 100% surface coverage) of the ligands on the NP's surface and the etching of surface defects. Since ligand exchange is a key mechanism in the silanization process, it is important that the affinity of amino ligands to the QDs' surfaces allows a successful substitution with TEOS molecules. It was shown that if the affinity of the initial ligands to the QD's surface is too high (when, for example, thiolated ligands are used as stabilizers), the possibility of ligand exchange is extremely low, which prohibits successful silica incorporation[19].

It is important to mention that the structure of the QDs also affects the fluorescent properties of the QD@SiO_2 NPs. More specifically, the thinner the wider band gap semiconductor shell, the higher the deterioration of the fluorescent properties of silica covered NPs. The synthesized QD^{550} probably do not have a uniform ZnS shell to protect the CdSe core from oxidation, thus the fluorescent properties of such QDs covered with silica shell quickly deteriorate. Even during one day of storage in a water solution, the QY immediately drops from an initial 50% down to 10% and keeps on decreasing with further storage. It may be safely assumed that the low temperature does not allow the ZnS lattice to properly build onto the CdSe core, due to the considerable differences in lattice parameters.

In the meantime, the SILAR technology, owing to the high temperature of the shelling process and the intermediate CdS layer, allows better protection of the fluorescent CdSe core and prevents a significant drop of the QY after silanization. The synthesized QD^{630}, with an initial QY of 36%, shows a QY of 22% after silica covering. In the case of QD^{600}, an alloy layer of CdZnS was added in between the CdS and ZnS shells. It was shown before[20] that addition of such a layer between these shells allows retaining the original QY after the silanization process, which was confirmed by our results (Table 1). QD^{600} with an intermediate CdZnS shell, synthesized according to the method presented in the experimental part, exhibits a relatively small QY drop from 53% to 43%, and it remains stable even after several months of storage.

Table 1. QYs of the synthesized QDs with different amount of shells, before and after silanization.

	Quantum Yield, %		
	QDs in toluene	**QD@SiO_2 freshly prepared**	**QD@SiO_2 after 1 month of storage**
QD^{550} CdSe/ZnS	50	10	-
QD^{600} CdSe/CdS/CdZnS/ZnS	53	43	41
QD^{630} CdSe/CdS/ZnS	36	22	19

4. CONCLUSIONS

Green, orange and red emitting CdSe-based QDs were synthesized by varying the number and the material of the wider-band gap shells. In order to receive stable water solutions, all three of them were coated with silica shells by means of a reverse microemulsion method. The fluorescent properties of the QDs were characterized before and after silanization. It was shown that the structure of the QD influences the QY of the silanized QDs: the more shells the QD has, the better the QY. Thus, silanization is a convenient route towards the synthesis of water-soluble, stable, bright QD-based labels with different emission colors, yielding a promising outlook for multiplex analysis.

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